

Tuexenia 38: 405–418. Göttingen 2018.  
doi: 10.14471/2018.38.013, available online at [www.zobodat.at](http://www.zobodat.at)



## Restoration possibilities of dry grasslands afforested by pine: the role of seed bank and remnant vegetation

### Restaurationsmöglichkeiten von mit Kiefern aufgeforsteten Steppenrasen: Rolle der Samenbank und Steppenrasen-Restvegetation

Anna Szabó & Eszter Ruprecht\*

*Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Republicii street 42,  
400015 Cluj-Napoca, Romania*

*\*Corresponding author, e-mail: [eszter.ruprecht@ubbcluj.ro](mailto:eszter.ruprecht@ubbcluj.ro)*

#### Abstract

*Stipa*-dominated steppe-like dry grasslands represent a great nature conservation priority in Romania and in the whole European Union. In Romania, due to their low productivity, many sites had been afforested by pines or black locust trees at the end of the 1950's in order to raise economic value and to hinder landslides on the steep slopes. Economic expectations did not live up, thus afforested sites will increasingly be targeted for grassland restoration, especially in protected areas of the Natura 2000 network. We analysed seed bank and aboveground vegetation in a 40-year-old black pine plantation site established on a former dry grassland near Viişoara (Transylvanian Lowland), and compared to that of an adjacent intact dry grassland stand. In both stands, eight 5 m × 5 m plots for soil and vegetation analysis were established. Inside each plot, six cylinder-shaped soil cores of 4 cm diameter and 10 cm depths were drilled; soil samples from the same plot were subsequently mixed up. Soil sampling was carried out in early spring, 2008. We analysed seed densities and composition in soil samples using the seedling emergence method. In addition, we recorded percentage cover of plant species in the plots in June. We wanted to find out on what extent we can rely on the soil seed bank and remnant vegetation, if active interventions are planned to restore a grassland stand in the place of a pine plantation. In total, 293 seedlings belonging to 51 species have emerged from soil samples. Seed density in the soil seed bank of the pine plantation was substantially higher ( $3500 \pm 2059$  seeds m<sup>-2</sup>, 0–10 cm) than that of the intact grassland ( $1360 \pm 469$  seeds m<sup>-2</sup>, 0–10 cm). Our results showed that the seed bank of the plantation site, although not specifically species rich, may still contain a considerable amount of target species. In addition, we found strong populations of grassland species in the understory of the plantation as remnants of the former vegetation, which, together with seeds in the seed bank can ensure a good starting point to a successful natural regeneration after tree removal. Due to the presence of ruderal species in the seed bank and shrubs in the aboveground vegetation, light restoration measures comprising weed- and shrub control are necessary following the complete removal of trees. We propose the re-introduction of grazing in order to ensure the spontaneous regeneration by propagule transport to restoration sites.

**Keywords:** afforestation, natural regeneration, Romania, seedling emergence, shrub encroachment, steppe, *Stipa*, Transylvanian Lowland

**Erweiterte deutsche Zusammenfassung am Ende des Artikels**

## 1. Introduction

Steppe-like grasslands represent a great conservation interest in the European Union (EUROPEAN COMMISSION 2007, GAFTA & MOUNTFORD 2008), mainly because they harbour many plant species of eastern and southern origin as well as several endemic taxa. In addition, they are considered to be relic vegetation types from the Late Glacial that escaped Holocene woodland invasions (ILLYÉS & BÖLÖNI 2007, KUNEŠ et al. 2008). Hence their protection is in the focus of conservation authorities. In Central-Eastern Europe they are usually situated in agricultural landscapes on southern or south-western facing slopes. During the last two decades of the 19<sup>th</sup> century an intensive campaign had begun for the forestation of steep slopes covered by low productivity steppe grasslands, at first with black locust (*Robinia pseudoacacia*), and later (from the beginning of the 20<sup>th</sup> century) with black and Scots pine (*Pinus nigra* and *P. sylvestris*) (TRACI et al. 1966, RESMERIȚĂ et al. 1968). Pine plantations were set to hinder landslides and to raise economic value of steep and often overgrazed slopes (RESMERIȚĂ et al. 1968).

Since pine plantations established on dry grasslands still harbour species of community interest, they often were included in protected areas (RUPRECHT et al. 2009). Due to the objectives set by the current conservation policy on one hand, and economic fail of pine plantation maintenance on the other hand, plantations will increasingly be targeted for grassland restoration, especially in protected areas of the Natura 2000 network.

Surveys performed in pine plantation sites reported a detrimental effect of planted trees on the original species pool and community structure of grasslands (e.g., through decreased light availability, increased nutrient content and leaf litter, and increased water table by trees) (LEEGE & MURPHY 2001, TAMÁS 2001, CREMENE et al. 2005, RUPRECHT et al. 2009). There are very few studies comparing seed bank density and composition between the vegetation of plantations (former grasslands) and intact grasslands. These studies report seed bank species impoverishment along with seed density decline in plantations as compared to grasslands (e.g., MACCHERINI & DE DOMINICIS 2003, CSONTOS 2007). The degree of species loss and seed bank depletion is apparently related to stand age (KALAMEES & ZOBEL 1997, BISTEAU & MAHY 2005), but also the habitat type seems to have an important role.

Although most of the seed bank studies conducted with restoration purpose agree that grassland restoration cannot rely or not exclusively rely on seed bank stores (BOSSUYT & HONNAY 2008), in some cases seed bank can have an important role especially in the initial phases of restoration (LIU et al. 2009, KALAMEES et al. 2012). Moreover, seed banks are able to preserve the seeds of threatened species (GODEFROID et al. 2011), worth to be considered during the restoration process. In addition, remnants of the former grassland vegetation, plant populations surviving the afforestation, can also have a very important role in the regeneration of such sites (MACCHERINI & DE DOMINICIS 2003). Feather grass (*Stipa* spp.) dominated dry steppe-like grassland is an important habitat type for nature conservation, and degraded stands are potential targets for future restoration actions in Romania. Thus, it is important to have information about their seed bank and vegetation composition in order to find out on what extent restoration efforts can rely on the seed bank and on remnants of the former vegetation. Based on the results of studies in other dry grasslands dominated by *Stipa* species (e.g., GHERMANDI 1997) or related dry grasslands (TÓTH & HÜSE 2014, KISS et al. 2016), we can expect a low representation of typical grassland species in the seed bank of our studied vegetation type (but see ZHAN et al. 2007).

In this study we aimed to explore the seed bank density and composition together with the composition of the aboveground vegetation in a 40-year-old black pine plantation, which was established on a dry grassland. In order to estimate the restoration potential in black pine plantations, we compared their vegetation attributes with that of an adjacent intact grassland having similar abiotic conditions. In the comparison we especially focused on typical grassland species. Our main questions were the following: (1) what is the seed bank composition and density in the pine plantation as compared to that of the intact grassland?; (2) what is the amount of target species groups in the seed bank and in the aboveground vegetation of the pine plantation?; and by this means (3) on what extent can we rely on the soil seed bank and on remnant aboveground vegetation, if active interventions are planned in order to restore a grassland stand in the place of a pine plantation? As encroachment of woody and weed species is a potential problem often compromising grassland restoration (CREMENE et al. 2005), we also assessed the proportion of such species in the seed bank and aboveground vegetation of the pine plantation.

## 2. Study area

The study was carried out in a dry grassland site and an adjacent pine plantation near Viișoara (“Coasta Lunii”, 46.5309 N, 23.9387 E; 330–340 m a.s.l.), situated in the western part of the Transylvanian Lowland (Câmpia Transilvaniei), Romania. The study site was designated owing to its prominent nature conservation value and representative species pool, evidenced by a former survey (RUPRECHT et al. 2009).

The bedrock consists of mixed Tortonian sediments (clay, marl and sand), on which carbonated chernozems were formed. The climate is temperate continental, with the mean annual precipitation around 590 mm, and maximum precipitation occurring during summer (June, July, and August). The mean annual temperature is 8.5 °C.

The study sites are located on a steep, south-western facing slope (average inclination 35°), covered originally by highly species-rich continental steppe-like grassland, which constitutes the priority habitat type 62CO (Ponto-Sarmatic steppes) of the Habitats Directive of the European Union. At the end of the 1950’s an intensive forestation with European black pine and Scots pine was implemented in the area, pine trees were planted over thousands of hectares of these grasslands. At present pine forests occupy most parts of the slopes and dry grasslands forms only small isolated remnants surrounded by a forest matrix. We have chosen one black pine plantation and one adjacent grassland stand for analysing and comparing seed bank and vegetation composition. Both stands were approximately 4 ha large. In the Pine plantation, trees were 10–14 m high, and canopy cover varied between 30–65%. Although grazing was forbidden in plantations, irregular sheep grazing could have occurred. The grassland stand was dominated by *Stipa pulcherrima*, and it was traditionally managed as a pasture for sheep. Management has gradually been abandoned and ceased around 2005, though occasional sheep grazing was observed in the area after 2005.

## 3. Methods

### 3.1 Seed bank and vegetation sampling

In both stands (pine plantation and grassland), eight plots for soil and vegetation analysis of 5 m × 5 m were established randomly in homogenous vegetation, excluding the fringe zone of the stands. Thus, the distance between a grassland plot and its plantation pair varied between 60 and

200 meters. Soil sampling was carried out in early spring (March 2008), before the start of germination and beginning of fresh seed shed. Inside each plot, six randomly placed cylinder-shaped soil cores of 4 cm diameter and 10 cm depths were drilled with a bakelite tube after removing the litter. Soil samples from the same plot were subsequently mixed up. Accordingly, an area of 0.0075 m<sup>2</sup> was sampled per each plot. Sample concentration by washing the soil samples through sieves of different mesh sizes (4 mm, 0.25 mm and 0.16 mm) was performed to reduce sample volume and to promote germination (TER HEERDT et al. 1996).

Concentrated soil samples were spread out to a thickness of 0.5–1 cm in plastic trays formerly filled with commercial potting soil and a 0.5 cm sand layer above the potting soil. Sand layer served to separate potting soil from our soil samples, thus we could easily mix the soil samples to facilitate germination of seeds after the summer resting period (see below). Five trays with commercial potting soil and with a sand layer on the top were used as control to account for the seed input from outside. In March, trays were placed in an outside experimental area in the University Botanical Garden of Cluj-Napoca covered with a thin transparent cloth put on the top of each frame to prevent high seed contamination; trays were watered regularly.

We analysed seed densities and composition in soil samples using the seedling emergence method (ROBERTS 1981). Seedlings were examined every two weeks between March and October 2008, counted and identified as soon as possible after germination (CSAPODY 1968, MULLER 1978). Unidentifiable seedlings were transplanted into pots and grown until they could be determined. After three months (in July), when no further seedling emergence was observed, the samples were placed in a dark room and left without watering for four weeks, after that they were resettled in the original outside experimental area. Seedling mortality during the whole study period was very low, only five seedlings (three from the plantation and two from the grassland seed bank) died before they could be determined. These seedlings were taken into account only in seed density calculations, but were excluded from further analysis. There were four *Populus canescens* seedlings emerging from the control trays, thus we decided to exclude the single *P. canescens* seedling, which emerged from our soil samples.

In order to have data about the composition of aboveground vegetation as well, we recorded percentage cover of both woody and herbaceous plant species in the plots in June 2008.

### 3.2 Data analysis

We compared the total species richness of the above ground vegetation and seed bank between the two stands (pine plantation and grassland). For the quantitative comparison, average seed densities were calculated for both stands. In addition, we calculated the similarity in species composition between the seed bank and aboveground vegetation for the two stands using the Jaccard index (KENT & COKER 1992).

For the calculations aiming to analyse the species composition of both the aboveground vegetation and the seed bank, species were classified in four main groups based on their habitat affiliation (SANDA et al. 1983): dry grassland specialists (species confined to *Festuco-Brometea* Br.-Bl. et Tx. ex Soó 1947 and *Koelerio-Corynephoretea canescentis* Klika in Klika et Novák 1941 class); general grassland species (occurring in a wide range of semi-natural and degraded grassland types); forest and scrubland species (*Quercu-Fagetea sylvaticae* Br.-Bl. et Vlieger in Vlieger 1937, *Quercetea pubescentis* Doing-Kraft ex Scamoni et Passarge 1959, *Trifolio-Geranietea sanguinei* T. Müller 1962, *Epilobietea angustifolii* Tx. et Preising ex von Rochow 1951, *Rhamno-Prunetea* Rivas Goday et Borja Carbonell 1961); and ruderal species (*Stellarietea mediae* Tx. et al. in Tx. 1950, *Artemisietea vulgaris* Lohmeyer et al. in Tx. 1950) (see Supplement E1). The proportion of species belonging to these four groups was compared between the seed bank and vegetation of the two stands by one-way analysis of variance (ANOVA).

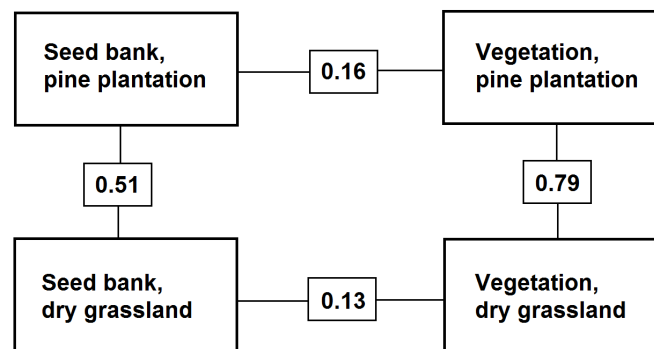
Statistical analyses were performed using the program Statistica, version 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA). Plant nomenclature follows Flora Europaea (TUTIN et al. 1964–1980).

## 4. Results

### 4.1 Seed bank density and composition in the pine plantation and intact grassland

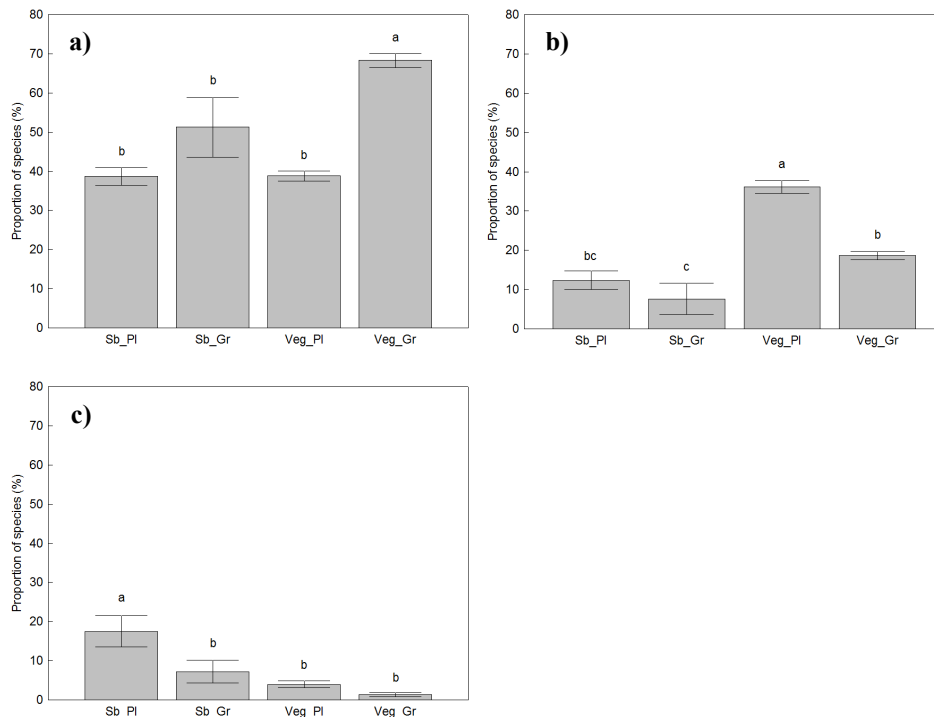
In total, 293 seedlings have emerged from the soil samples originating from the two sites. Seed density in the soil seed bank of the pine plantation ( $3,500 \pm 2,059$  seeds  $m^{-2}$ , 0–10 cm) was substantially higher than that of the intact grassland ( $1,360 \pm 469$  seeds  $m^{-2}$ , 0–10 cm).

A total number of 51 species emerged from the two seed bank sample-series. The seed bank of the pine plantation was more species rich than that of the intact grassland, since we found the seeds of 44 species in the soil samples originating from the plantation and 25 in the soil samples of the intact grassland. Grassland and pine plantation seed banks had only 17 species in common, and the Jaccard similarity between the composition of the two seed banks was 0.51 (Fig. 1). There was no difference in the proportion of dry grassland specialists (Fig. 2a), general grassland species, forest and scrubland species (Fig. 2b) found in the seed bank of the pine plantation and the intact grassland, but ruderal species were more abundant in the seed bank of the plantation (Fig. 2c). It is worth mentioning that some rare grassland species emerged from the seed bank samples of the pine plantation, e.g., *Peucedanum tauricum*, *Carduus hamulosus*, *Astragalus dasyanthus* and *Veronica prostrata* (see Supplement E1). Among the five most abundant species in the seed bank of the pine plantation there were four dry grassland specialists and only one ruderal species (*Erigeron annuus*), the same as in the intact grassland (Fig. 3). In addition, there was a considerable overlap in the most abundant species of the seed bank of the two sites (Fig. 3). It is important to notice that the dominant species of the intact grassland (*S. pulcherrima*) had a very low density in the seed bank of the intact grassland (only 1 seedling emerged from our seed bank samples).



**Fig. 1.** Similarity in species composition between the soil seed bank and vegetation for the pine plantation and intact grassland, as measured by the Jaccard index.

**Abb. 1.** Ähnlichkeit der Artenzusammensetzung zwischen Samenbank und Vegetation eines Kiefernforsts und Steppenrasens. Die angegebenen Werte sind Jaccard-Indices.



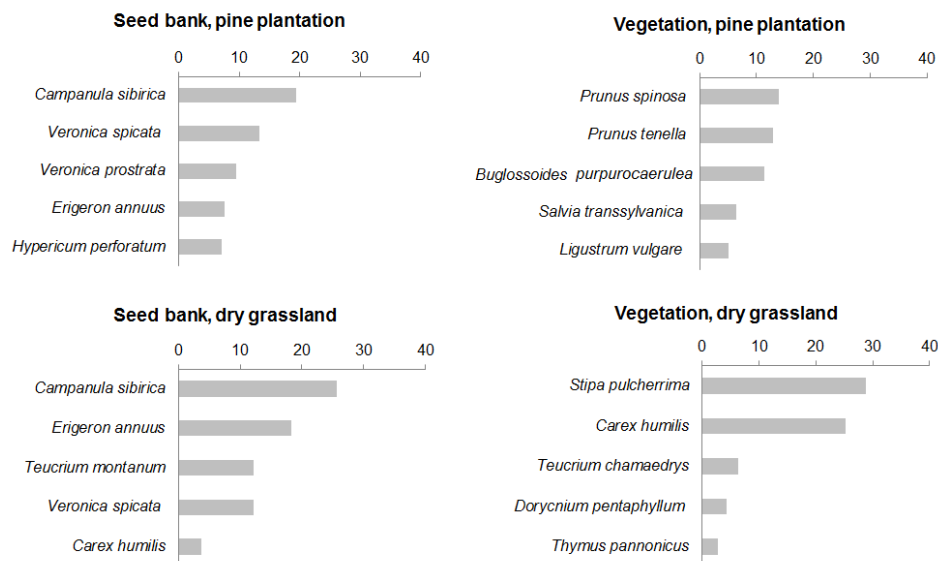
**Fig. 2.** Proportion of specific species groups in the seed bank (Sb\_PI – plantation seed bank, Sb\_Gr – grassland seed bank) and vegetation (Veg\_PI – vegetation plantation, Veg\_Gr – vegetation grassland). Values are means and standard errors. Different letters denote significant differences at  $p < 0.05$  as obtained by the Tukey post-hoc test. **a)** Dry grassland specialists. Results of a one-way ANOVA ( $MS = 1627.1$ ,  $df = 3$ ,  $F = 11.94$ ,  $p = 0.0001$ ). **b)** Forest and scrubland species. Results of a one-way ANOVA ( $MS = 1251.2$ ,  $df = 3$ ,  $F = 24.2$ ,  $p = 0.001$ ). **c)** Ruderal species. Results of a one-way ANOVA ( $MS = 401$ ,  $df = 3$ ,  $F = 7.88$ ,  $p = 0.001$ ).

**Abb. 2.** Anteile spezifischer Artengruppen in der Samenbank (Sb) und Vegetation (Veg) eines Kiefernforsts (PI) und Steppenrasens (Gr). Dargestellt sind Mittelwerte und Standardfehler. Mittelwerte mit unterschiedlichen Buchstaben unterscheiden sich signifikant ( $p < 0,05$ ) nach einem Tukey-Post-hoc-Test. **a)** Steppenrasenspezialisten Die Ergebnisse der einfachen ANOVA lauten:  $MS = 1627,1$ ;  $df = 3$ ;  $F = 11,94$ ;  $p = 0,0001$ . **b)** Wald- und Buschlandarten. Die Ergebnisse der einfachen ANOVA lauten:  $MS = 1251,2$ ;  $df = 3$ ;  $F = 24,2$ ;  $p = 0,001$ . **c)** Ruderalarten. Die Ergebnisse der einfachen ANOVA lauten:  $MS = 401$ ;  $df = 3$ ;  $F = 7,88$ ;  $p = 0,001$ .

#### 4.2 Comparison of the seed bank and vegetation of the pine plantation and intact grassland

We found 106 plant species in total (within the eight sample plots) in the aboveground vegetation of the pine plantation and 80 in the intact grassland, with 71% of the species present in both sites.

Similarity in species composition between the seed bank and aboveground vegetation was very low, 0.16 in the case of the plantation and 0.13 for the intact grassland (Fig. 1). By contrast, similarity in species composition of the vegetation between the grassland and plantation was quite high (0.79).



**Fig. 3.** The first five most dominant species in the seed bank and vegetation of the pine plantation and dry grassland. Bars represent mean relative seed densities (seed bank) and mean relative cover values (vegetation).

**Abb. 3.** Die fünf häufigsten Arten in der Samenbank und Vegetation des Kiefernforsts und Steppenrausens. Die beiden linken Graphen zeigen den durchschnittlichen Anteil einer Art in der Samenbank und die beiden rechten Graphen deren relative Deckung in der Vegetation.

In total, 89 target species (dry grassland specialists and general grassland species) were present in the seed bank and aboveground vegetation of the pine plantation. These species were well represented in the aboveground vegetation of the intact grassland (68%,  $31 \pm 2$  sp/plot and 22%,  $10 \pm 3$  sp/plot, Fig. 2a). They were present in significantly lower numbers in the aboveground vegetation of the pine plantation, but their proportion was still representative (38%,  $20 \pm 4$  sp/plot and 38%,  $20 \pm 4$  sp/plot, Fig. 2a). However, the species number of these groups was significantly lower in the seed bank of the plantation, their proportion was still high in the seed bank stores (39%,  $5 \pm 1$  sp/plot for dry grassland specialists and 37%,  $5 \pm 3$  sp/plot for general grassland species), as compared to the intact grassland (51%,  $3 \pm 2$  sp/plot and 36%,  $2 \pm 1$  sp/plot, respectively, Fig. 2a).

Even though, many forest and scrubland species were present in the aboveground vegetation of the plantation site, this group was poorly represented in the seed bank (Fig. 2b). In turn, while ruderal species were very few in the vegetation (both in the plantation and intact grassland), they appeared in moderate proportion in the seed bank of the pine plantation, but not in that of the intact grassland (Fig. 2c).

When we compared vegetation composition based on the most dominant species, the difference between the plantation and grassland was obvious. In the pine plantation, forest and scrubland species were among the most dominant, while in the intact grassland there were only grassland species among the five most dominant species (Fig. 3).

## 5. Discussion

### 5.1 Seed bank composition of the intact dry steppe-like grassland and pine plantation

Our results showed that seed bank density in the studied dry steppe-like grassland (1,360 seeds m<sup>-2</sup>, 0–10 cm) is similar to seed densities found in other, Western-European dry grasslands (770 seeds m<sup>-2</sup>, 0–20 cm, WILLEMS & BIK 1998; 930 seeds m<sup>-2</sup>, 0–5 cm, BOSSUYT et al. 2006; 1,984 seeds m<sup>-2</sup>, 0–5 cm, KALAMEES & ZOBEL 1997), but it is considerably lower than seed densities found in Central-European dry grasslands (e.g., loess grasslands in Hungary, 20,200 seeds m<sup>-2</sup>, 0–10 cm, or xerothermic grasslands in Poland, 1,642–21,217 seeds m<sup>-2</sup>, 0–5 cm, KISS et al. 2016). At the same time, species richness of the seed bank (25 species in total) was found to be substantially lower than the values reported from other dry grasslands (KALAMEES & ZOBEL 1997, KALAMEES et al. 2012, KISS et al. 2016). Taking into account the low density of grassland seed bank in most ecosystems (see e.g., BOSSUYT & HONNAY 2008, KISS et al. 2016) and the high spatial heterogeneity in the seed bank distribution of most species (CZARNECKA 2004, BOSSUYT et al. 2007), a more intensive sampling (higher volume of sampled soil and higher sample number) would have revealed higher species richness in case of the studied steppe-like grassland system (see RUPRECHT & SZABÓ 2012). Surprisingly, in the case of the pine plantation site we found higher seed densities (3,500 seeds m<sup>-2</sup>, 0–10 cm) and higher seed species richness (44 species) than in the intact grassland (25 species). Other studies have reported quite the opposite trend, seed bank depletion in sites afforested both by pines and black locust (CSONTOS et al. 1997, MACCHERINI & DE DOMINICIS 2003, MATUS et al. 2003, BOSSUYT et al. 2006, BOSSUYT & HONNAY 2008). This trend can be explained mostly by the lower seed bank density characteristic for forest ecosystems (BOSSUYT & HONNAY 2008). In this regard, our plantation site has a seed bank density typical for scattered planted sites (MACCHERINI & DE DOMINICIS 2003) or scrub vegetation (BOSSUYT et al. 2006). A similar enrichment and concentration of seed bank stores was found by KALAMEES & ZOBEL (1997) in alvar grasslands overgrown with young pine forest. In their opinion dormancy induced by the canopy cover of trees (shading effect) and litter accumulation are responsible for seed density increase in such sites. We have also found a thick litter layer (4.37 cm on average) in the pine plantation composed mostly of pine leaves.

We found very low similarity in species composition between seed bank and above-ground vegetation both for the intact grassland site and pine plantation, which is a quite general trend showed by other studies as well (BEKKER et al. 1997, DAVIES & WAITE 1998, KISS et al. 2016). In the case of the grassland site, one of the reasons for this low similarity may be that perennial species with high cover in the vegetation (e.g., *Carex humilis*, *Teucrium chamaedrys*, *Dorycnium pentaphyllum* subsp. *herbaceum*, *Salvia transsylvanica*; Fig. 3, Supplement E1), and especially the dominant species (*S. pulcherrima*), had low seed density in the seed bank. *Stipa pulcherrima* has transient seeds (CSONTOS 2001), which may be the explanation for the poor representation of its seeds in the soil seed bank. This is in line with our former study on the same vegetation type, where we have found that grassland and ruderal species poorly represented in the vegetation, but with high seed production and persistent seeds, were the most abundant in the seed bank (e.g., *Veronica spicata* subsp. *orchidea*, *Campanula sibirica*, *Conyza canadensis*, *Hypericum perforatum* and *Verbascum phoeniceum*, RUPRECHT & SZABÓ 2012). Interestingly, in feather grass dominated steppes in Inner Mongolia of China ZHAN et al (2007) found high abundance of *Stipa krylovii* and other perennial species' seeds in the seed bank of grazed and enclosed steppe stands.



In the case of the pine plantation, the low proportion of forest and scrubland species and the moderate representation of ruderal species in the seed bank, which were almost completely lacking from the aboveground vegetation, were responsible for the low similarity. Another explanation for the low similarity between the seed bank and aboveground vegetation is that the seeds of rare species are difficult to detect in the soil, due to their low number and uneven distribution (THOMPSON et al. 1997). In our case, even by a relatively low intensity sampling, we could detect the seeds of some of the rare grassland species in the seed bank of the pine plantation, which is important from a restoration perspective.

## 5.2 Regeneration possibilities of the pine plantation on dry grassland after tree removal

An interesting finding of our study is that the seed bank of a pine plantation established on a steppe-like grassland even after 40 years may still contain a considerable amount of grassland species (16 species, similar to that of an intact grassland). This can partly ensure the future natural regeneration of grasslands in the place of plantations, if trees are removed. In addition, grassland species, remnants of the former dry grassland vegetation were present in the understory of pine plantation. E.g., strong populations of *Salvia transsylvanica*, *Carex humilis*, and *Teucrium chamaedrys*, dry grassland specialists and general grassland species, were still present nonetheless abiotic and biotic conditions have been drastically changed (shading, more mesic conditions, competition by trees and shrubs) due to tree planting. Survival of these populations as well as the maintenance of grassland species' seeds in the seed bank was probably promoted by the continuous seed influx from the adjacent small grassland patches, which were not planted, thus could remain intact. That is the reason for the high similarity in species composition of both the vegetation and seed bank between the pine plantation and studied dry grassland site (Fig. 1).

Contrary to the high similarity in species composition with the intact grassland, in the plantation site there was a high cover of shrubs and a relatively high representation of scrubland and forest species. This can be attributed to differences in the abiotic conditions between the grassland and plantation site. Thus, canopy shading by planted trees and low competition microsites, characteristic for dry grasslands of low vegetation cover, have probably facilitated the establishment and encroachment of shrubs in the plantation sites. This phenomenon was also reported by other studies on plantations established on open grassland (KALAMEES & ZOBEL 1997), and can be considered a more general scenario taking place in the whole Transylvanian region (RUPRECHT et al. 2009).

As the invasion of weed species is a potential problem, which often compromise grassland restorations (e.g., CREMENE et al. 2005, TÖRÖK et al. 2010, SZITÁR et al. 2014), we assessed whether weed species are present in the seed bank or vegetation of the pine plantation, and found out that they represent a moderate proportion (17.5%) of the species pool of seed bank. Even so, in case of restoration interventions aiming at recreating dry grasslands in the place of plantations, weed control is necessary.

## 6. Conclusions and conservation remarks

Our study confirmed that remnants of former vegetation in the understory of black pine plantations established on dry steppe-like grassland and seeds of target species in their seed bank can potentially support the successful spontaneous grassland regeneration following the removal of woody vegetation (e.g., DÖLLE & SCHMIDT 2009, LIU et al. 2009). Since shrub encroachment may be typical for plantation sites, post-restoration management comprising

the removal of resprouting woody species is necessary following the complete removal of trees (MICHIELSEN et al. 2017). In plantations on steep slopes, it is very important to remove the trees and shrubs gradually, during an approximately 5 year period in order to avoid soil erosion or the dangerous landslides. In addition, since ruderal species may be abundant in the seed bank, weed suppression is necessary in the first phase of restoration (TÖRÖK et al. 2012). Reintroduction of grazing may be a good solution both for weed control and for the suppression of shrubs. Moreover, grazing may support grassland recovery by propagule transport between intact grassland stands in the surroundings and the restoration site (COSYNS et al. 2005, KUITERS & HUISKES 2010).

## Erweiterte deutsche Zusammenfassung

**Einleitung** – Aufforstungen wirken sich meistens auf die Artenzusammensetzung und Artenvielfalt sowie Struktur der ursprünglichen Vegetation aus (LEEGE & MURPHY 2001, TAMÁS 2001, CREMENE et al. 2005, RUPRECHT et al. 2009). Obwohl die meisten Studien über Restaurationen zu dem Ergebnis kommen, dass die Wiederherstellung der ursprünglichen Vegetation von Aufforstungsflächen nicht alleine durch die Samenbank im Boden erfolgen kann (BOSSUYT & HONNAY 2008), so spielt die Samenbank zumindest in der Initialphase der Restauration doch eine wichtige Rolle (LIU et al. 2009, KALAMEES et al. 2012). Zudem können Samenbanken die Diasporen von bedrohten Arten enthalten (GODEFROID et al. 2011). *Stipa*/Federgras-Steppenrasen stellen aus Naturschutzsicht wertvolle Lebensräume dar, und durch Aufforstungen degradierte Bestände bilden potenzielle Objekte für Restaurationen. Um einen möglichen Restaurationserfolg solcher degradierter Aufforstungsflächen über die Samenbank abschätzen zu können, sind zunächst genaue Kenntnisse der Samenbank nötig. Basierend auf bereits vorliegenden Ergebnissen für *Stipa*-dominierte Steppenrasen (GHERMANDI 1997) oder verwandte Trockenrasen (TÓTH & HÜSE 2014, KISS et al. 2016), kann man in der Samenbank dieses Vegetationstyps eher mit einer geringen Anzahl typischer Arten rechnen (siehe jedoch ZHAN et al. 2007). In diese Studie untersuchten wir die Samenbank eines vor etwa 40 Jahren mit Schwarz-Kiefern (*Pinus nigra*) aufgeforsteten Steppenrasens und verglichen diese mit der Samenbank eines benachbarten, nicht aufgeforsteten Steppenrasens. Zusätzlich wurde die aktuelle Vegetation des Kiefernforsts und rezenten Steppenrasens untersucht. Unsere Hauptfragen lauten: (1) Welche Arten und Samenmengen sind in der Samenbank des Kiefernforsts und rezenten Steppenrasens vorhanden? (2) Welche Steppenrasenarten sind noch in der aktuellen Vegetation des Kiefernforsts vorhanden? (3) Inwieweit könnte nach Entfernung der Kiefern eine Wiederherstellung der ehemaligen Steppenrasenvegetation über die Samenbank möglich sein?

**Untersuchungsgebiet** – Das Untersuchungsgebiet liegt nahe Viișoara in West-Transsilvanien in Rumänien. Die Untersuchungsflächen liegen an einem steilen Südwesthang. Dieser war ursprünglich mit ausgedehnten artenreichen Steppenrasen bewachsen, wurde jedoch in den 1950-er Jahren in weiten Teilen mit Schwarz-Kiefern aufgeforstet. Untersucht wurden ein solcher Kiefernforst und ein benachbarter rezenter Steppenrasen.

**Material und Methoden** – In dem Kiefernforst und rezenten Steppenrasen wurden im Frühjahr 2008 jeweils acht 5 m × 5 m-Probeflächen eingerichtet. Nachdem die Streuschicht entfernt worden war, wurden in jeder Probefläche an sechs zufällig gewählten Stellen zylinderförmige Bodenbohrkerne von 4 cm Durchmesser und 10 cm Länge (Tiefe) gezogen und pro Probefläche zu einer Mischprobe vereint. Die Samenbank dieser Bodenproben wurde mit der Auflaufmethode bestimmt. Zusätzlich wurde die Vegetation der Flächen im Juli 2008 aufgenommen. Die Jaccard-Ähnlichkeit der Samenbank und Vegetation wurde bestimmt. Zusätzlich wurden die Anteile von vier ökologischen Artengruppen (Steppenrasenspezialisten, Graslandgeneralisten, Ruderalarten, Wald- und Buschlandarten) sowohl in der Samenbank als auch in der Vegetation des Kiefernforsts und Steppenrasens bestimmt und mit ANOVA und einem Post-hoc-Test verglichen.

**Ergebnisse** – Insgesamt konnten wir in den Bodenproben 293 Sämlinge von 51 Arten nachweisen. In der Samenbank des Kiefernforsts lag die Samendichte mit  $3500 \pm 2059$  Samen/m<sup>2</sup> wesentlich höher als im Steppenrasen ( $1360 \pm 469$  Samen/m<sup>2</sup>). Die Jaccard-Ähnlichkeit der Samenbanken des Kiefernforsts und Steppenrasens lag bei 0,51 (Abb. 1). Lediglich 17 Arten kamen sowohl in der Samenbank des Kiefernforsts als auch in der des Steppenrasens vor. Die Prozentsätze der Steppenrasenspezialisten sowie Wald- und Buschlandarten in der Samenbank unterschieden sich nicht signifikant zwischen Kiefernforst und Steppenrasen (Abb. 2a und 2b). Allerdings waren in der Samenbank des Kiefernforsts mehr Ruderalarten als in der des Steppenrasens vorhanden (Abb. 2c). In der Samenbank des Steppenrasens waren sowohl Steppenrasenspezialisten als auch Graslandgeneralisten stark vorhanden. In der Vegetation waren diese Arten dagegen im Kiefernforst deutlich schwächer als im Steppenrasen vorhanden (22 % vs. 68 % bzw.  $10 \pm 3$  Arten/25m<sup>2</sup> vs.  $31 \pm 2$  Arten/25m<sup>2</sup>, Abb. 2a). Viele Wald- und Buschlandarten waren im Kiefernforst nur in der Vegetation und nicht in der Samenbank vorhanden (Abb. 2b). Umgekehrt waren im Kiefernforst viele Ruderalarten v. a. in der Samenbank aber kaum in der Vegetation vorhanden (Abb. 2c).

**Diskussion** – Die Menge der Samen in der Samenbank unseres Steppenrasens lag mit 1360 Samen/m<sup>2</sup> bis in 10 cm Bodentiefe ähnlich hoch wie in westeuropäischen Trockenrasen (WILLEMS & BIK 1998: 770 Samen/m<sup>2</sup> bis in 20 cm Tiefe; BOSSUYT et al. 2006: 930 Samen/m<sup>2</sup> bis in 5 cm Tiefe; KALAMEES & ZOBEL 1997: 1984 Samen/m<sup>2</sup> bis in 5 cm Tiefe). Sie lag jedoch wesentlich niedriger als in mitteleuropäischen Trockenrasen, z. B. ungarischen Lössrasen mit 20.200 Samen/m<sup>2</sup> bis in 10 cm Bodentiefe oder polnischen Trockenrasen mit 1642–21.217 Samen/m<sup>2</sup> bis in 5 cm Tiefe (KISS et al. 2016). Überraschenderweise stellten wir in der Samenbank des Kiefernforsts sowohl eine höhere Samenmenge (3500 Samen/m<sup>2</sup> bis in 10 cm Tiefe) als auch höhere Artenzahl (44 vs. 25 Arten) fest als in der des Steppenrasens. Andere Studien fanden umgekehrte Ergebnisse, was eher auf eine Abnahme der Samenbank mit der Zeit in aufgeforsteten Trockenrasen hindeutet (CSONTOS et al. 1997, MACCHERINI & DE DOMINICIS 2003, MATUS et al. 2003, BOSSUYT et al. 2006, BOSSUYT & HONNAY 2008). Diese Abnahme kann auch durch die allgemein niedrigere Samenbankdichte in Wald-Ökosystemen erklärt werden (BOSSUYT & HONNAY 2008). Die Anzahl der Samen in der Samenbank unseren Kiefernforsts lag in einem für Aufforstungen (vgl. MACCHERINI & DE DOMINICIS 2003) oder Buschländer (vgl. BOSSUYT et al. 2006) typischen Bereich. Eine ähnliche Anreicherung der Samenbank wurde von KALAMEES & ZOBEL (1997) in einem mit Kiefern überwachsen Alvar-Grasland festgestellt. Ein bemerkenswertes Ergebnis unserer Studie war, dass die Samenbank des vor etwa 40 Jahren mit Kiefern aufgeforsteten Steppenrasens immer noch 16 Graslandarten enthält; das sind ähnlich viele, wie in dem rezenten Steppenrasen. Einige dieser Graslandarten waren aber auch in der rezenten Vegetation des Kiefernforsts vorhanden; daher stellt hier ihre Präsenz in der Samenbank wohl kein Überbleibsel der ursprünglichen Vegetation dar. Vermutlich stellt auch die Präsenz anderer Graslandarten in der Samenbank des Kiefernforsts kein Überbleibsel aus der Zeit vor der Aufforstung dar, sondern ist vielmehr einen kontinuierlichen Eintrag von Samen aus den benachbarten Steppenrasen geschuldet. Insgesamt stellten wir in der Samenbank und Vegetation des Kiefernforsts 89 Arten fest. Diese Zahl erscheint uns ein vielversprechender Ausgangspunkt für die Wiederherstellung der ursprünglichen Steppenrasenvegetation – falls die Kiefern entfernt werden sollten. Allerdings wandern in frische Rodungsflächen oftmals Straucharten ein, die eine mögliche Restauration stören könnten und nur durch Wiederaufnahme der Landnutzung reduziert werden können bzw. manuell entfernt werden müssen (MICHIELSEN et al. 2017). Insgesamt erscheint uns die Wiederherstellung des Steppenrasens aus der Samenbank in Kombination mit der Steppenrasen-Restvegetation möglich, wenn die Kiefern entfernt werden.

## Acknowledgements

We are very grateful to the “Alexandru Borza” Botanical Garden at Cluj-Napoca for ensuring the necessary infrastructure during the germination study. We also thank Annamária Fenesi and two undergraduate students, Gabriella Benze and Blanka Gyurkóczy, for their help during the field work. We thank Thomas Kuhn for the translation of German abstract and Thomas Becker for corrections and final editing. We thank the two reviewers for constructive comments and Balázs Deák for his editorial atten-

tion and suggestions to the text. This work was supported by CNCSIS-UEFISCSU, project PN II-RU TE 296 nr. 71/29.07.2010 and the Szülőföld Fund Hungary (Scholarship Program for Research Abroad). During writing the manuscript, ER was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

## Supplements

**Additional supporting information may be found in the online version of this article.**

**Zusätzliche unterstützende Information ist in der Online-Version dieses Artikels zu finden.**

**Supplement E1.** List of plant species present in vegetation and emerging from seed bank samples of the pine plantation and intact grassland, mean vegetation cover, mean seed density, and frequency in seedbank samples.

**Anhang E1.** Liste der in der Vegetation vorhandenen und aus den Samenbankproben keimenden Pflanzenarten des Kiefernforsts und des intakten Steppenrasens, mittlere Vegetationsdeckung, Frequenz in den Vegetationsplots, mittlere Samendichte, und Frequenz in den Samenbankproben.

## References

- BEKKER, M., VERWEIJ, G.L., SMITH, R.E.N., REINE, R., BAKKER, J.P. & SCHNEIDER, S. (1997): Soil seed bank in European grasslands: does land use affect regeneration perspectives? – *J. Appl. Ecol.* 34: 1293–1310.
- BISTEAU, E. & MAHY, G. (2005): Vegetation and seed bank in calcareous grassland restored from a *Pinus* forest. – *Appl. Veg. Sci.* 8: 167–174.
- BOSSUYT, B., BUTAYE, J. & HONNAY, O. (2006): Seed bank composition of open and overgrown calcareous grassland soils – a case study from Southern Belgium. – *J. Environ. Manag.* 79: 364–371.
- BOSSUYT, B. & HONNAY, O. (2008): Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. – *J. Veg. Sci.* 19: 875–884.
- BOSSUYT, B., VAN WICHELEN, J. & HOFFMANN, M. (2007): Random soil seed bank sampling allows predicting seedling density but not species richness in situ – a case study of a drained lake bottom. – *J. Veg. Sci.* 18: 443–450.
- COSYNS E., CLAERBOUT S., LAMOOT I. & HOFFMANN M. (2005): Endozoochorous seed dispersal by cattle and horses in a spatially heterogeneous landscape. – *Plant Ecol.* 178: 149–162.
- CREMENE, C., GROZA, G., RAKOSY, L., SCHILENKO, A.A., ERHARDT, A. & BAUR, B. (2005): Alterations of steppe-like grasslands in Eastern Europe: a threat to regional biodiversity hotspots. – *Conserv. Biol.* 19: 1606–1618.
- CSAPODY, V. (1968): Keimlingsbestimmungsbuch der Dicotyledonen. – Akadémiai Kiadó, Budapest, Hungary: 286 pp.
- CSONTOS, P. (2001): A természetes magbank kutatásának módszerei (Methods for studying the natural seed bank) [in Hungarian]. – *Synbiologica Hungarica* 4, Scientia Kiadó, Budapest, Hungary.
- CSONTOS, P. (2007): Dolomityepepek magbankja ültetett feketefenyvesek talajában. (Relict seed bank of dolomite grasslands in the soil of *Pinus nigra* plantations) [in Hungarian with English summary]. – *Tájökológiai Lapok* 5: 117–129.
- CSONTOS, P., TAMÁS, J. & KALÁPOS, T. (1997): Soil seed banks and vegetation recovery on dolomite hills in Hungary. – *Acta Bot. Hung.* 40: 35–43.
- CZARNECKA, J. (2004): Microspatial structure of the seed bank of xerothermic grassland – Intra-community differentiation. – *Acta Soc. Bot. Pol.* 73: 155–164.
- DAVIES, A. & WAITE, S. (1998): The persistence of calcareous grassland species in the soil seed bank under developing and establishes scrub. – *Plant Ecol.* 136: 27–39.
- DÖLLE, M. & SCHMIDT, W. (2009): The relationship between soil seed bank, above-ground vegetation and disturbance intensity on old-field successional permanent plots. – *Appl. Veg. Sci.* 12: 415–428.

- EUROPEAN COMMISSION (2007): Interpretation Manual of European Union Habitats – EUR27. – European Commission, DG Environment, Brussels: 144 pp.
- GAFTA, D. & MOUNTFORD, J.O. (Eds.) (2008): Manual de interpretare a habitatelor Natura 2000 din România (Manual for interpreting Natura 2000 habitats from Romania) [in Romanian]. – Risoprint, Cluj, Romania: 101 pp.
- GHERMANDI, L. (1997): Seasonal patterns in the seed bank of a grassland in north-western Patagonia. – *J. Arid Environ.* 35: 215–224.
- GODEFROID, S., RIVIÈRE, S., WALDREN, S., BORETOS, N., EASTWOOD, R. & VANDERBORGHT, T. (2011): To what extent are threatened European plant species conserved in seed banks? – *Biol. Conserv.* 144: 1494–1498.
- ILLYÉS, E. & BÖLÖNI, J. (Eds.) (2007): Lejtősztyepek, löszgyepek és erdőssztyeprétek Magyarországon (Slope steppes, loess steppes and forest steppe meadows in Hungary) [in Hungarian]. – Budapest, Hungary: 236 pp.
- KALAMEES, R., PÜSSA, K., ZOBEL, K. & ZOBEL, M. (2012): Restoration potential of the persistent soil seed bank in successional calcareous (alvar) grasslands in Estonia. – *Appl. Veg. Sci.* 15: 208–218.
- KALAMEES, R. & ZOBEL, M. (1997): The seed bank in an estonian calcareous grassland: comparison of different successional stages. – *Folia Geobot.* 32: 1–14.
- KENT, M. & COKER, P. (1992): *Vegetation Description and Analysis. A Practical Approach.* – Belhaven Press, London, UK: 363 pp.
- KISS, R., VALKÓ, O., TÓTHMÉRÉSZ, B. & TÖRÖK, P. (2016): Seed bank research in Central-European grasslands - An overview. – In: MURPHY, J. (Ed.): *Seed Banks: Types, Roles and Research:* 1–34. Nova Science Publishers, New York.
- KUITERS, A.T. & HUISKES, H.P.J. (2010): Potential of endozoochorous seed dispersal by sheep in calcareous grasslands: correlations with seed traits. – *Appl. Veg. Sci.* 13: 163–172.
- KUNEŠ, P., PELÁNKOVÁ, B., CHYTRÝ, M., JANKOVSKÁ, V., POKORNÝ, P. & PETR, L. (2008): Interpretation of the last-glacial vegetation of eastern-central Europe using modern analogues from southern Siberia. – *J. Biogeogr.* 35: 2223–2236.
- LEEGE, L.M. & MURPHY, P.G. (2001): Ecological effects of the non-native *Pinus nigra* on sand dune communities. – *Can. J. Bot.* 79: 429–437.
- LIU, M., JIANG, G., YU, S., LI, Y. & LI, G. (2009): The role of soil seed banks in natural restoration of the degraded Hunshandak Sandlands, Northern China. – *Restor. Ecol.* 17: 127–136.
- MACCHERINI, S. & DE DOMINICIS, V. (2003): Germinable soil seed-bank of former grassland converted to coniferous plantation. – *Ecol. Res.* 18: 739–751.
- MATUS, G., TÓTHMÉRÉSZ, B. & PAPP, M. (2003): Restoration prospects of abandoned species-rich sandy grassland in Hungary. – *Appl. Veg. Sci.* 6: 169–178.
- MICHIELSEN, M., SZEMÁK, L., FENESI, A., NIJS, I. & RUPRECHT, E. (2017): Resprouting of woody species encroaching temperate European grasslands after cutting and burning. – *Appl. Veg. Sci.* 20: 388–396.
- MULLER, F.M. (1978): *Seedlings of the North-Western European Lowland. A flora of seedlings.* – Dr W. Junk B.V. Publishers, The Hague, Boston: 654 pp.
- RESMERIȚĂ, I., CSŪRÖS, I. & SPÎRCHEZ, Z. (1968): *Vegetația, ecologia și potențialul productiv pe versanții din Podișul Transilvaniei (Vegetation, ecology and production potential on hillslopes of the Transylvanian Basin)* [in Romanian]. – Ed. Academiei R.S.R., București, Romania: 206 pp.
- ROBERTS, H.A. (1981): Seed banks in soil. – *Adv. Appl. Biol.* 6: 1–55.
- RUPRECHT, E. & SZABÓ, A. (2012): – Grass litter is a natural seed trap in long-term undisturbed grassland. – *J. Veg. Sci.* 23: 495–504.
- RUPRECHT, E., SZABÓ, A., ENYEDI, M. & DENGELER, J. (2009): Steppe-like grasslands in Transylvania (Romania): characterisation and influence of management on species diversity and composition. – *Tuexenia* 29: 353–368.
- SANDA, V., POPESCU, A., DOLTU, M.I. & DONIȚĂ, N. (1983): *Caracterizarea ecologică și fitocenologică a speciilor spontane din flora României (Ecological and fitosociological characteristics of the native flora of Romania)* [in Romanian]. – *Stud. Comun. Suppl.* 25: 5–126.
- SZITÁR, K., ÓNODI, G., SOMAY, L., PÁNDI, I., KUCS, P. & KRÖEL-DULAY, G. (2014): Recovery of inland sand dune grasslands following the removal of alien pine plantation. – *Biol. Conserv.* 171: 52–60.

- TAMÁS, J. (2001): A feketefenyvesek telepítése Magyarországon, különös tekintettel a dolomitkopár-  
okra (Austrian pine plantations in Hungary with special attention to dolomite hills) [in Hungarian  
with English summary]. – Természetvédelmi Közlem 9: 75–85.
- TER HEERDT, G.N.J., VERWEIJ, G.L., BEKKER, R.M. & BAKKER, J.P. (1996): An improved method for  
seed bank analysis: seedling emergence after removing the soil by sieving. – *Funct. Ecol.* 10:  
144–151.
- THOMPSON, K., BAKKER, J.P. & BEKKER, R.M. (1997): Soil seed banks of North West Europe:  
Methodology, density and longevity. – Cambridge University Press, Cambridge, UK: 276 pp.
- TÓTH, K. & HÜSE, B. (2014): Soil seed bank in loess grasslands and their role in grassland recovery. –  
*Appl. Ecol. Env. Res.* 12: 537–547.
- TÖRÖK, P., DEÁK, B., VIDA, E., VALKÓ, O., LENGYEL, S. & TÓTHMÉRÉSZ, B. (2010): Restoring grass-  
land biodiversity: sowing low diversity seed mixtures can lead to rapid favourable changes. – *Biol.*  
*Conserv.* 143: 806–812.
- TÖRÖK, P., MIGLÉCZ, T., VALKÓ, O., KELEMEN, A., DEÁK, B., LENGYEL, S. & TÓTHMÉRÉSZ, B.  
(2012): Recovery of native grass biodiversity by sowing on former croplands: Is weed suppression  
a feasible goal for grassland restoration? – *J. Nat. Conserv.* 20: 41–48.
- TRACI, C. & COSTIN, E. (1966): Terenurile degradate și valorificarea lor pe cale forestieră. (Degraded  
soils and their rehabilitation by means of forestry) [in Romanian]. – Edit. Agro-Silvică, București,  
Romania: 283 pp.
- TUTIN, T.G., HEYWOOD, V.H., BURGESS, N.A. et al. (1964–1993): *Flora Europaea*, Vols. 1–5. – Cam-  
bridge University Press, Cambridge, UK.
- WILLEMS, J.H. & BIK, L.P.M. (1998): Restoration of high species density in calcareous grassland: the  
role of seed rain and soil seed bank. – *Appl. Veg. Sci.* 1: 91–100.
- ZHAN, X., LI, L. & CHENG, W. (2007): Restoration of *Stipa krylovii* steppes in Inner Mongolia of  
China: Assessment of seed banks and vegetation composition. – *J. Arid Environ.* 68: 298–307.